

## Nonlethal, attenuated, transfusion-associated graft-versus-host disease in an immunocompromised child: case report and review of the literature

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**BACKGROUND:** Transfusion-associated graft-versus-host disease (TA-GVHD) is a rare complication of transfusion of nonirradiated blood components. It usually affects children in high-risk groups, including those who have primary immunodeficiencies (PIDs). It usually presents with skin, hepatic, digestive, and hematologic involvement and is normally fatal.

**CASE REPORT:** We report the case of a nonlethal, attenuated, TA-GVHD in a 7-month-old boy. The disease was marked by the presence of a severe rash but lacked all the other usual manifestations. We speculate that the unusually benign course of this disease, which has normally a fulminant course, was due to the fact that this child was under high-dose corticotherapy at the time of the engraftment. This fortunate coincidence led to the survival of this child and allowed the diagnosis of a combined immunodeficiency.

**CONCLUSION:** A high index of suspicion is required for the diagnosis and proper management of PID. The administration of nonirradiated blood components in the first year of life, sometimes before the clinical suspicion of a PID, is of great concern. TA-GVHD may be more prevalent than reported in the literature and it is possibly a nonidentified cause of death in recipients with unexplained death and nondiagnosed PID.

**T**ransfusion-associated graft-versus-host disease (TA-GVHD) is a rare complication of blood transfusion.<sup>1</sup> Its true incidence is not known but it is estimated that it is decreasing with the routine application of the prevention techniques, such as the gamma irradiation of cellular blood products with doses of 25 to 50 Gy for transfusions in recipients belonging to risk groups<sup>2</sup> and leukoreduction (with much better performance with the recent advances of the filtration technology) of all blood components.<sup>3</sup> The “threshold” dose of lymphocytes required for TA-GVHD in humans is not known and probably depends on the recipient’s ability to reject the transfused lymphocytes.<sup>1</sup> As little as  $3 \times 10^4$  T lymphocytes is sufficient to cause lethal TA-GVHD in marrow transplant recipients.<sup>4</sup> It has been stated that gamma irradiation with 25 Gy is adequate to cause T-cell depletion and prevent T-cell growth.<sup>5</sup>

No cases of TA-GVHD were reported in 2008 in the United Kingdom and there was only one report since the implementation of leukoreduction of all blood components (except granulocytes) in 1999.<sup>6</sup> Nevertheless, TA-GVHD might still be underreported<sup>7</sup> because it can mimic many other conditions, such as viral infections or drug reaction, and it has normally a fulminant course,<sup>8</sup> thus not permitting a proper diagnosis. Adding to this, the current understanding is that there is growing evidence that supports the possibility of the occurrence of

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**ABBREVIATIONS:** CID = combined immunodeficiency; PICU = pediatric intensive care unit; PID(s) = primary immunodeficiency(-ies); TA-GVHD = transfusion-associated graft-versus-host disease.

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**TABLE 1. Risk factors for TA-GVHD**

Significantly increased risk	No risk
Autologous marrow/stem cell transplant recipients	Healthy newborns
Allogeneic marrow/stem cell transplant recipients	Patients with AIDS
Hodgkin's disease	Acute leukemia without transplantation
B-cell malignancies (non-Hodgkin's lymphoma, multiple myeloma, Waldenström's macroglobulinemia, ALL)	Aplastic anemia
Fludarabine, cladribine therapy	
Granulocyte transfusions in infants	
Intrauterine transfusions	
Directed donations from blood relatives	
HLA-matched platelets	
Congenital immunodeficiency disorders	

subclinical TA-GVHD, especially among small children and among those who receive cellular transfusions after traumatic injury.<sup>9</sup>

In order that TA-GVHD becomes a reality, as described by Billingham in 1966,<sup>10</sup> several conditions must be observed: 1) immunologic competent lymphocytes must engraft, 2) the host must differ antigenically from the graft, and 3) the recipient must not be able to mount an adequate response against the graft. Because the first two premises are almost universally present, the third is the one that spots the difference and based on it risk groups<sup>11</sup> were created for the development of TA-GVHD (Table 1). A similar result may be observed when there is a HLA similarity between the donor and the recipient (like in family member donors or in populations that have a high homogeneity in HLA types), even in the absence of immunosuppression as the haploidentical donor lymphocytes may escape the recipient's immune surveillance and attack the recipient's tissues by reacting against HLAs that are not shared.<sup>8</sup> Newborns are at particular risk of TA-GVHD because they are physiologically immune incompetents and underlying congenital T-cell immunodeficiencies may be yet undiagnosed. It is recommended that all blood for intrauterine transfusions and exchange transfusions should be irradiated. Interestingly, there is no indication for the irradiation of blood for routine transfusions of the term or preterm newborn.<sup>1</sup>

## MATERIALS AND METHODS

### Preparation of red blood cells

Using anticoagulant citrate dextrose, the red blood cells (RBCs) were separated using buffy coat procedure and stored with saline-adenine-glucose-mannitol (376 mOsm/L).

### DNA polymorphism analysis

Evaluation of chimerism was performed on four short-tandem repeats locus (HUMTH01, HUMVWA,

HUMF13A1; HUMFES and a sex marker amelogenin)-specific polymerase chain reaction (PCR) amplification with dye primers. Donor and recipient were characterized and the degree of mixture was evaluated before and after cell separation using a cell sorter (Aria, BD, Franklin Lakes, NJ). DNA was extracted using M48 DNA extraction apparatus (QIAgen, Chatsworth, CA) and short-tandem repeat amplification was performed in each cell subset using the primers mentioned. The degree of chimerism was analyzed after capillary array electrophoresis, in an automatic

DNA sequencing machine (Beckman Coulter, Fullerton, CA), with software (CEQ8000, Beckman Coulter), as described elsewhere.<sup>12</sup>

### Immunologic testing

Lymphocyte proliferation responses to phytohemagglutinin, concanavalin A, and pokeweed mitogen were assayed as previously reported.<sup>13</sup>

### Review of the literature

We searched PubMed for articles in English published between 1980 and August 2009 with the following key words in the title: "transfusion-associated graft-versus-host." We found 151 articles, some descriptions of nonlethal cases but no case of attenuated TA-GVHD in a child.

## CASE REPORT

A 7-month-old boy with normal development and growth, as well as no known past infections, was admitted in the pediatric intensive care unit (PICU) of a tertiary pediatric hospital in Lisbon for pneumococcal sepsis complicating an adenovirus bronchiolitis. He needed mechanical ventilation for 7 days. On Days 2 and 7, under 4 mg/kg/day hydrocortisone, he received two transfusions of 10 mL/kg leukoreduced, nonirradiated RBCs (aged 0 and 5 days) from the same donor. On Day 14 he started to have fever and developed a generalized rash with coalescent erythematous macules involving palms and soles, treated as toxic reaction to drugs with 3 mg/kg/day methylprednisolone. All potential drugs involved were discontinued (namely vancomycin and cefotaxime). The child was discharged to the general pediatrics ward on Day 23 where he stayed until Day 40. His stay in the general pediatrics ward was marked by severe dependence on oxygen and worsening of the rash, with areas of erythematous papules and scaling (Fig. 1). On Day 40 he was readmitted in the PICU for severe rash, sepsis, and respiratory failure. At this time,



Fig. 1. Skin involvement.

TABLE 2. Immunology workup

Laboratory test	Result	Reference value
Serum immunoglobulins		
IgG (mg/mL)	4.64	3.3-6.2
IgM (mg/mL)	0.38	0.2-0.8
IgA (mg/mL)	<0.2	0.5-1.3
IgE (U/mL)	17	<30
Vaccine titers		
Tetanus (IU/mL)	0.95	>0.15
Diphtheria (IU/mL)	0.12	>0.15
Isohemagglutinin titers A and B	<8	>16
Lymphocyte subsets		
CD3+ cells/ $\mu$ L	660	2156-3413
CD4+ cells/ $\mu$ L	418	1360-3066
CD4CD45RA (%)	60	75-90
CD8+ cells/ $\mu$ L	231	560-1803
CD19+ cells/ $\mu$ L	385	811-1792
CD16+56+ cells/ $\mu$ L	55	164-801
Lymphocyte proliferation (cpm)		
Phytohemagglutinin	3164 $\pm$ 511	
Concanavalin A	2755 $\pm$ 527	
Pokeweed mitogen	7890 $\pm$ 358	
V $\beta$ TCR repertoire	Normal	

he was under no medication except prednisolone. Viral PCR and shell viral culture for enterovirus; Epstein-Barr virus; cytomegalovirus; human herpesvirus 6, 7, and 8; varicella zoster virus; jc virus; and BK virus were negative. During this admission, according to the history of severe infections caused by both virus and bacteria, a clinical suspicion of combined immunodeficiency (CID) and GVHD was raised. The immunologic panel confirmed the hypothesis of a CID/hypomorphe SCID and the most common genetic defects were ruled out (Table 2).

The child started antibacterial and antifungal prophylaxis, as well as immunoglobulin replacement. Clinically he did very well, apart from two septic events related with the central venous catheter (*Staphylococcus aureus* and *Klebsiella pneumoniae*).

The chimerism studies confirmed the presence of exogenous lymphocytes accounting for 16% of total lym-

phocyte count. Maternal engraftment was ruled out and the lymphocytes were shown to belong to the RBC transfusion donor (Fig. 2). This fact along with the clinical manifestations (the characteristic rash but lacking digestive, hepatic, and hematologic involvement) and the skin biopsy (lymphocyte infiltrate with focal parakeratosis and necrosis of the keratinocytes, without eosinophil infiltration) led to the diagnosis of attenuated TA-GVHD. Unfortunately, in situ hybridization of the skin biopsy that could have identified the donor lymphocytes was not performed. He started treatment with cyclosporine (serum levels of 150-250 ng/mL), allowing the weaning of the corticotherapy. There was a slow but progressive clinical improvement with disappearance of the rash along with the disappearance of the chimerism (1 month after the beginning of this therapy the chimerism showed the absence of exogenous lymphocytes). At the age of 14 months he was discharged from the PICU, still under cyclosporine and corticotherapy without any TA-GVHD manifestations.

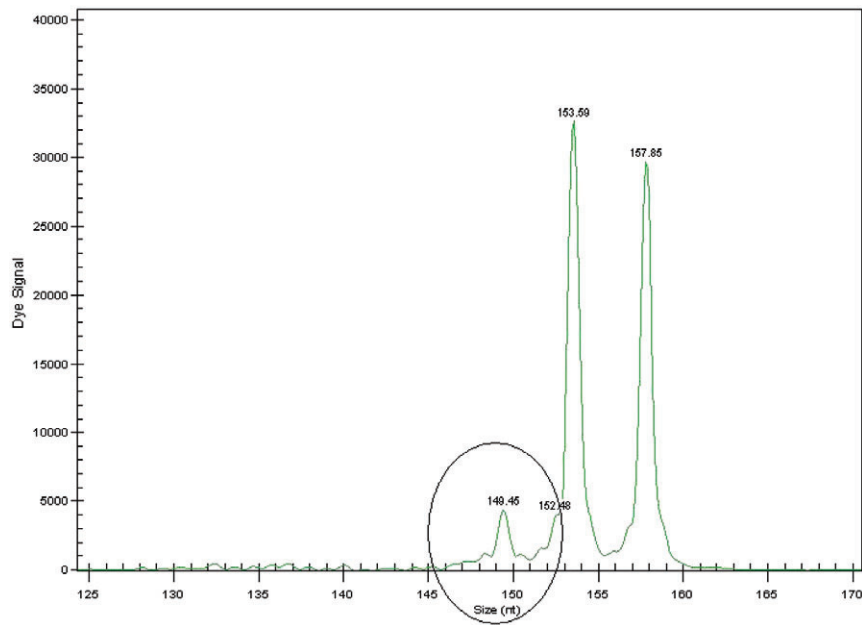
## DISCUSSION AND CONCLUSION

TA-GVHD is, as stated above, a fatal complication of blood transfusion. Its rarity is probably due to the improvement of the prevention techniques but presumably also to underdiagnosis.<sup>7</sup> The disease usually presents 4 to 21 days (median, 10 days) after the transfusion of any cellular blood product and has a fulminant course with skin, hepatic, digestive, and hematologic involvement.<sup>14</sup> The classic symptoms are fever, rash, liver dysfunction, and diarrhea. Most commonly, fever is the first manifestation and it is followed by the appearance of an erythematous, maculopapular skin rash that starts on the trunk and spreads through palms and soles. The degree of liver involvement is variable, as are the gastrointestinal complications. Pancytopenia develops in a median of 16 days and is severe.

The definitive diagnosis of TA-GVHD needs the demonstration of donor-derived cells or DNA fragments in the blood or affected tissues of the recipient. Nevertheless, we underline that the diagnosis of TA-GVHD cannot be based only in the presence of lymphocytes of the blood transfusion donor as there can exist as much as 1% of subclinical microchimerism.<sup>9</sup>

Although it is rare, we speculate that some cases might be classified as toxic, viral, or autoimmune occurrences with fatal outcome in toddlers. In the United Kingdom 13 cases of TA-GVHD have been reported since 1996 with a mortality of 100%.<sup>6</sup> To our knowledge, no cases of TA-GVHD have been reported in Portugal since 2000.

Aware of its rarity and knowing the difficulties that diagnosing this condition raise, the authors believe that this child had subclinical TA-GVHD. The diagnosis was based not only on the engraftment of exogenous



**Fig. 2. Chimerism studies.**

lymphocytes but also on the fact that the recipient was immunodeficient and had characteristic rash and skin biopsy,<sup>15</sup> and other causes such as viral infections or drug reaction were ruled out.

The absence of all the other clinical characteristics is extremely infrequent and is possibly linked to the fact that he was receiving high-dose corticotherapy for his respiratory condition. Its mild course permitted the diagnosis of a CID. In pediatric practice, apart from the newborn period and oncology, the most important factor for the presence of TA-GVHD is primary immunodeficiency (PID). Among these, the risk is variable and almost exclusive of the cellular PID.<sup>1</sup>

A high index of suspicion is required for the diagnosis and proper management of PID. The administration of nonirradiated blood components in the first year of life, sometimes before the clinical suspicion of a PID, is of great concern. TA-GVHD is probably more prevalent than reported in the literature and it is possibly a nonidentified cause of death in recipients with unexplained death and nondiagnosed PID.

In countries where the diagnosis of cellular PID (especially severe CID) is well below expected (in conformity with the expected occurrence), the possibility of a fatal outcome due to the transfusion of nonirradiated blood components before the suspicion of cellular PID is a reality. Some institutions have therefore decided to irradiate all blood that is transfused in the first year of life.

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#### CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest relevant to the manuscript submitted to **TRANSFUSION**.

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